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MONITORING OF PESTICIDES CHRONIC TOXICITY AMONG EXPOSED WORKERS BY USING EFFECT BIOMARKERS

***Hamdi Abdulwahab Alhakimi, [1]Anas Yahya Alghamdi and [2]Ahmad Hamza Alabbasi, Omar Abdulaziz Alzahrani[3] and Abdullah Sinhat Alsahli[4]**

*Assistant Professor, Albaha University, Saudi Arabia.
[1,2]GP at Security Forces Health Center
[3]GP at Security Forces Health Center in Riyadh
[4]Medical Doctor at Security Forces Hospital

Corresponding Author Dr. Hamdi Abdulwahab Alhakimi
Assistant Professor, Albaha University, Saudi Arabia

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ABSTRACT

Background: Workers who regularly use pesticides at increasing risk of pesticides toxicity. Monitoring of pesticides chronic toxicity is necessary to prevent long term health hazards. This review aimed to compare the validity of cholinesterase biomarkers to that of cytogenetic biomarkers in assessment of pesticides chronic toxicity. **Methods:** Medline and Cochrane library are searched electronically to collect studies which conducted cholinesterase and cytogenetic biomarkers simultaneously among pesticides exposed workers. A total of 1249 papers have found. After excluding of irrelevant, ineligible, duplicate and very low quality papers, only 16 studies are included in this systematic review. As a gold standard test was absent, we had to validate these biomarkers in order to compare them together. Validation had two phases, phase1 the ability of biomarkers to detect a significant difference between pesticides exposed and non-exposed workers. Phase 2 is purposed to determine a dose-response relationship and to control for confounding. **Results:** at the study level, the cytogenetic biomarkers have a higher reliability (100%) than cholinesterase biomarkers (75%) for detection of statistically significant difference between chronically exposed and non exposed workers. Furthermore, within studies using cholinesterase biomarkers, erythrocyte cholinesterase (E chE) has higher reliability (78%) than plasma cholinesterase (P AchE) (62%). There is a significant association between duration of exposure and results of cytogenetic biomarkers. This association found to be less evident between duration of exposure and (E chE), and it is absent between duration of exposure and (P AchE). **Conclusion:** The cytogenetic biomarkers have more reliability than cholinesterase biomarkers in assessment of chronic toxicity caused by pesticides exposure.

KEYWORDS: cholinesterase, cytogenetic, Medline and Cochrane.

BACKGROUND

All pesticides should be toxic to be effective against the pests they are targeting. In the other hand this toxicity may cause harm to human who get in contact with them. Many farmers and industrial workers at increasing risk of pesticides toxicity because they get in contact with pesticides in their routine work. There are two types of toxicity acute and chronic. The chronic toxicity is an effect of long repeated exposure for small doses of pesticides rather than a large single dose. Therefore, monitoring of pesticides chronic toxicity is necessary to avoid long term health hazards like hepatic, renal problems and cancer.[1-4] .Effect biomarkers, such as cholinesterase and cytogenetic, have been known to be appropriate measures to monitor for toxic effects of pesticides among exposed workers.[5,6]This review aimed to compare the validity of cholinesterase biomarkers to that of cytogenetic biomarkers in assessment of pesticides chronic toxicity Also, it attempted to investigate the association between the duration of pesticides exposure and the resultant toxic effects.

METHODS

This review focused in studies that measuring both cytogenetic and cholinesterase biomarkers at the same time as indicators for pesticides chronic toxicity among their participants. We compared the validity of these biomarkers at study level not at the individual level. As there is no gold standard method, we had to conduct a biomarkers validation before we started to compare these biomarkers. Biomarkers validation has to main phases. Phase 1 aimed to determine the reliability of a biomarker to detect a statistically significant difference between exposed and non-exposed workers, after that we compared the reliability of cholinesterase biomarkers with that of cytogenetic biomarkers. Phase 2 mainly purposed to determine the dose-response relationship between pesticides and their biomarkers with controlling

for the effect of confounders. Pesticides dose is consisted of two main elements, the intensity of exposure and the duration of this exposure. In this review, because no sufficient information found about intensity of pesticides exposure in included studies, we restricted this evaluation to the association between biomarkers measurement and duration of pesticides exposure. After that, we compare the available evidence of association that found between cytogenetic biomarkers to that of cholinesterase biomarkers.

Concerning confounding factors, we had two groups of confounders. The first group includes factors that confound the comparison between biomarkers which we control for them by simultaneous assessment of biomarkers in the same participants. The second group of factors is that confounding the relation between exposed and non exposed workers which should be already controlled for in the methodology of included studies. (Tables of assessment of bias of included studies in appendices).

Types of outcome measures

While biomarkers are predictive assays rather than diagnostic, they are used as proxy indicators for chronic pesticides toxicity in our review. The outcome that we looked for is a detection of the significant difference between pesticides exposed and non-exposed workers which is considered as a positive result in the context of our review. In other hand absence of a significant difference considered as a negative result.

Four cytogenetic biomarkers and tow acetylecholine biomarkers have been evaluated. Cytogenetic biomarkers are chromatid aberrasions (CA's), mononuclei (MN), sister chromatid exchange (SChE) and Commet Assay (CA). Cholinesterase biomarkers are plasma cholinesterase (Pch E) and erythrocyte (Ech E) cholinesterase. Regarding the association between biomarkers and duration of exposure, we were interested in presence of a significant correlation with reported values of correlation coefficient (r) if available.

Keywords and search strategy

They are demonstrated by summary of search results (Table 1). The flow of the information through the different stages of a systematic review (identification, screening, eligibility, inclusion) is demonstrated by Figure (1). Data were collected by Data Extraction forms (Tables of included study characteristics in appendices).

Quality appraisal

We conducted a critical appraisal by using quality assessment checklist (Table of quality assessment chicklist in appendices) focusing in key elements of observational studies. The final judgment on quality of studies is summarized in four domains (high, moderate, low or very low). Since we had only observational studies, no study is assessed to be high regarding quality issue. Therefore only moderate, low and very low are applicable in our review.

RESULTS

We searched internet (Medline, Google scholar and Cochrane Databases) at 10April 2012 looking for keywords demonstrated in table (1). A total of 1249 papers have found. After examining of paper titles and abstracts for relevancy and after excluding of duplicates only 25 papers have found to be eligible to the objective of this review. After that full text papers are retrieved for these 25 papers and examined for exclusion and inclusion criteria, only 16 have been applicable for these criteria. Also a quality assessment has done for these 16 papers by one observer and further three papers are excluded as they assessed to be very low quality papers.[7-9] Therefore, at the end only 13 studies have been included in this review. A flow diagram is shown in figure (1)

Quality Assessment of included studies

Only one observer has assessed methodological quality for 13 included studies by using the checklist that contains the key elements of observational studies (Table 2). Eight (62%) of all included studies have assessed to be with moderate quality and the remaining five studies (38%) have found to be low quality studies. As we assumed previously, since they are observational studies, no included study have evaluated as a high quality study.

Findings

Although there are 13 included studies, the study of Simoniello et al [10]has conducted on four groups of participants. Therefore it is actually consist of four minor studies and for each study we have measurement for one or more cytogenetic biomarkers and one or more cholinesterase biomarkers. So we can assume that there are 16 included studies during the calculations of the findings of this review (table 3).

Among theses 16 studies, the results of cytogenetic biomarkers were always indicate significant difference between pesticides exposed and non-exposed participants which we can consider it as (positive result) in the context of our review. Regarding cytogenetic biomarkers approximately all included studies reported positive results, although Remor et al.[11] used tow cytogenetic biomarkers (MN and CA) in the same participants and he found positive result for Commet Assay (CA) and negative result for Mononuclei biomarkers. Mononuclei biomarkers (MN) have used in five included studies which represents (31%) of all included studies. Also the chromosomal aberrations biomarkers (CA's) have been used in other five studies. Commet Assay biomarkers (CA) have used in nine studies (56%) of included studies where they have been used in six studies alone, in tow studies with (MN) and in one study with Chromosomal Aberrations (CA's). No included study has used Sister Chromatid Exchange (SChE).

The results of cholinesterase biomarkers were not such that consistent as in cytogenetic biomarkers. Erythrocyte Cholinesterase Esterase (E chE) have performed in nine (56%) of included studies. While it has done alone in four studies, it has performed in conjunction with Plasma Cholinesterase Esterase (P chE) among five other studies. Among these nine studies, seven studies (78%) have statistically significant differences between pesticides exposed and non-exposed workers (positive result). Plasma cholinesterase esterase (P chE) has conducted in thirteen studies (81%) of included studies in which eight of them (62%) reported statistically significant differences (positive result).

Therefore, these results indicate that cytogenetic biomarkers have more ability to detect positive results compared to cholinesterase biomarkers when there is a comparison between two groups with different history of pesticides exposure. In more technical terms, the studies using cytogenetic biomarkers have higher reliability than cholinesterase biomarkers for detection of statistical significant difference between chronically pesticides exposed and non-exposed workers. Furthermore, within studies using cholinesterase biomarkers, erythrocyte cholinesterase esterase (E chE) has more reliability than plasma cholinesterase (P chE) for detection of statistical significant differences between chronically exposed and non- exposed workers. Generally, total reliability was found to be 100% and 75% for cytogenetic and cholinesterase biomarkers respectively. Reliability for each biomarker separately is found in table (4).
Three studies have investigated the statistical correlation between cytogenetic and cholinesterase biomarkers. Naravaneni et al.[12] and Paz-y-Mino et al.[13] have observed significant correlation between cytogenetic biomarkers (CA and CA's) and cholinesterase biomarkers (E chE), while Singh et al.[14] reported that no significant correlation found between (CA) and (E chE) biomarkers.

The duration of exposure we found in this review is not a short duration; it is a long duration which may prolong for several years with minimum reported duration of six months Paz-y-Mino et al.[13] The mean duration of exposure are reported clearly in 8 studies. Other 8 studies, either used different statistics to describe the duration of exposure like range or equation, or they did not report anything at all. The mean of means for duration of exposure in these 8 studies have found to be 11.2 years (Table 5).

Bhalli ea al.[15] and Zeljezic et al.[16] have observed significant correlation between duration of exposure and results of cytogenetic biomarkers (MN and CA), whereas Naravaneni et al.[12] reported no significant correlation between the duration of exposure and cytogenetic biomarkers such as CA and CA's. Ali et al.[17] reported that the group of highest duration of exposure (more than15 years) have significant increase in cytogenetic biomarkers (MN) compared to lesser exposure groups (which are from 1 to 5, from 6 to 10 and from 11 to 15).

Both Cholinesterase biomarkers (E chE and P chE) have found to be correlated with duration of exposure by Naravaneni et al.[12] Also Singh et al [14] reported a significant negative correlation between duration of exposure and erythrocyte cholinesterase (E chE) biomarkers with ($r = -0.352$, $p < 0.05$).

Regarding occupation of study participants, most of included studies (75%) have conducted on farm workers like pesticides sprayers, mixers and flowers pickers. The remaining 4 studies (25%) are carried out in pesticides industrial workers and they tend to yield more detailed information about their participants such as duration of pesticides exposure and presence of correlations between different biomarkers. Types of pesticides for which study participants exposed are generally a mixture of pesticides more commonly organophosphates followed by carbamates and pyrethroids.

Table (1): Summary of search results

Database	Search Terms	Search Strategy	Papers
Medline	PESTICIDES (MESH) or pesticides (keyword). BIOMONITORING (MESH) or biomonitoring (keyword). CHOLINESTERASE (MESH) or cholinesterase (keyword). CYTOGENTIC BIOMARKER (MESH) or cytogenetic biomarker (keyword). . Genotoxicity (keyword). Micronuclei (keyword). Sister chromatid exchange (keyword). Chromosomal aberrations (keyword). Biological monitoring (keyword).		
	Search 1 **Search 2** **Search 3** **Search 4**	1 and 2 1 and 3 1 and 4 1 and 5	**89** **804** **13** **65**

	Search 5	1 and 6	**79**
	Search 6	1 and 7	**73**
	Search 7	1 and 8	**1**
	Search 8	1 and 9 and 3	**58**
	Search 9	1 and 9 and 4	**18**
	Search 10	1 and 9 and 6	**16**
	Search 11	1 and 9 and 7	**14**
	Search 12	8	**19**
Cochrane library	**Search 13**	Pesticides (keyword)	**0**
Total	**Titles and Abstracts examined** **Papers retrieved** **Papers included in review**		**1249** **25** **13**

Limits Activated: Humans, All Adult: 19+ years, Child: 6-12 years, Adolescent: 13-18 years

Table (2): Quality Assessment Checklist

Study Question **Study Population**	**Clearly focused and appropriate question** • **Description of study populations** • Sample size justification
Comparability of Subjects†	Specific inclusion/exclusion criteria for all groups • Criteria applied equally to all groups • Comparability of groups at baseline with regard to disease status and prognostic factors • Study groups comparable to non-participants with regard to confounding factors • *Use of concurrent controls* • Comparability of follow-up among groups at each assessment
Exposure or Intervention	**Clear definition of exposure** • Measurement method standard, valid and reliable • Exposure measured equally in all study groups
Outcome Measurement	
Primary/secondary outcomes clearly defined	• Outcomes assessed blind to exposure or intervention status • Method of outcome assessment standard, valid and reliable • Length of follow-up adequate for question
Statistical Analysis	Statistical tests appropriate • Multiple comparisons taken into consideration • Modeling and multivariate techniques appropriate • Power calculation provided • **Assessment of confounding** • Dose-response assessment, if appropriate
Results	• **Measure of effect for outcomes and appropriate measure of precision** • Adequacy of follow-up for each study group
Discussion	• **Conclusions supported by results with biases and limitations taken into consideration**
Funding or Sponsorship	• ***Type and sources of support for study***

Table (3): Summary of the findings

Study	Population	Cytogenetic biomarkers	Cholinesterase biomarkers	Number of exposed	Number of non-exposed	Grade of study quality
Ali-2008.[17]	farmers	+	+	69	69	moderate
Bhalli-2006.[15]	Industrial workers	+	+	29	35	moderate
Berga-1993.[21]	farmers	+	+	24	10	low
Carbonell-1995.[22]	farmers	+	-	29	29	low

Kunstadter-2006.[23]	farmers	+	+			low
Naravaneni-2007.[12]	farmers	+	+	210	160	moderate
Pastor-2002.[24]	farmers	+	-	39	22	moderate
Paz-y-Mino-2002.[13]	farmers	+	+	41	41	moderate
Remor-2009.[11]	farmers	+	+	37	20	low
Shadnia-2005.[25]	Industrial workers	+	-	21	21	low
Simoniello-2010.[10] group A (direct exposure) (indirect exposure) group B (direct exposure) (indirect exposure)	farmers	+	 +	 27 27 18 23	 A=30 B=20	moderate
Singh-2011.[14]	Industrial workers	+	+	70	70	moderate
Zeljezic-2007.[16]	Industrial workers	+	-	30	30	moderate

(+) means the study found a significant difference between exposed and non exposed workers.
(-) means the study did not find a significant difference between exposed and non exposed workers.
High quality: Further research is very unlikely to change our confidence in the study results.
Moderate quality: Further research is likely to have an important impact on our confidence in the study results and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the study results and is likely to change the estimate.
Very low quality: We are very uncertain about the study results.

Table (4): Reliability for each biomarker

Biomarker	Reliability %
Chromosomal Aberrations	100
Commet Assay	100
Mononuclei	94
Erythrocyte Cholinesterase	78
Plasma Cholinesterase	62

Table (5): Correlation between biomarkers and duration of pesticides exposure

Study	Cytogenetic biomarkers	Cholinesterase biomarkers		Correlation between biomarkers & duration of exposure
Ali-2008.[17]	+	+	10.26 + - 6.14	
Bhalli-2006.[15]	+	+	13.48 - 3.84	Significant correlation was found between duration of exposure and cytogenetic biomarkers.
Berga-1993.[21]	+	+	3.6 + - 2.7	
Carbonell-1995.[22]	+	-	not applicable	
Kunstadter-2006.[23]	+	+	no data	
Naravaneni-2007.[12]	+	+	4.5 + - 2.7	Significant correlation between Ach biomarkers and duration of exposure. (no significant correlation between duration of exposure and cytogenetic biomarkers)
Pastor-2002.[24]	+	-	8.31+ - 1.12	
Paz-y-Mino-2002 .[13]	+	+	6 to 66 months	
Remor-2009.[11]	+	+	25.69±10.14	
Shadnia-2005.[25]	+	-	8.1 years	
Simoniello-2010.[10]	+	+	no data	
Singh-2011.[14]	+	+	No data	Significant negative association was observed between AChE with duration of exposure ($r = -0.352$, $p < 0.05$).

Zeljezic-2007.[16]	+	-	15.7 years	there are correlation between cytogenetic biomarkers and duration of exposure)

(+) means the study found a significant difference between exposed and non exposed workers.
(-) means the study did not find a significant difference between exposed and non exposed workers

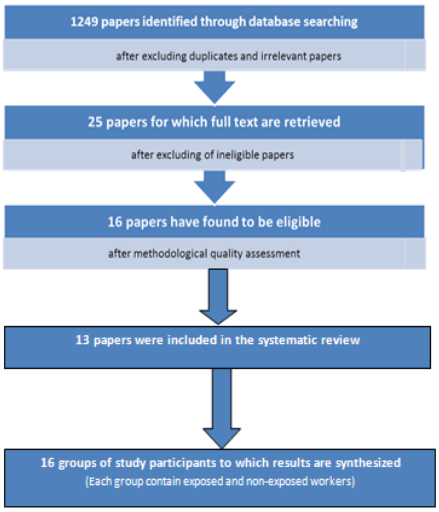

Figure (1): Flow diagram

DISCUSSION

The lack of validation of most biomarkers of effect is probably the most critical impediment to the broad use of biomarkers in risk assessment. In this case of absence of gold standard test, the process of validation has two phases as reported by Qu et al.[18] The main purpose of phase (1) was to determine whether these biomarkers could reliably detect differences between exposed and non-exposed participants, which are the minimal screening criterion for a biomarker. Phase (2) of the validation is mainly focused on evaluating the dose-response relationship and confounding factors.

In our review we aimed to compare the reliability of cytogenetic and cholinesterase biomarkers to detect the significant difference between pesticides exposed and non-exposed workers which represent phase (1). Then we assessed the association between the duration of exposure and these biomarkers which is a part of phase (2).

McMichael & Hall.[19] postulated that earlier results may be obtained from epidemiological studies if use of a biomarker increases the statistical power of the study which means the ability of study to detect the difference when it is true. During the discussion of the review findings we assuming that a real difference is already found between pesticides exposed and non exposed workers regarding biomarkers measurement. Thus the results of this review show that cytogenetic biomarkers have more reliability to detect a significant difference between exposed and non exposed workers than cholinesterase biomarkers.

The question which may arise, why is it as high as 100% of included studies reported significant difference between pesticides exposed and non-exposed workers by using a cytogenetic biomarkers (CA's and CA)?, or in other terms is there a possibility of false positive results to be reported by our included studies? The answer is, it could make sense to detect these significant differences by 100% of included studies because of no sufficient use of (PPE) by these workers (There is no study reported use of PPE) and prolonged pesticides exposure (mean of means for duration of exposure in these 8 studies have found to be 11.2 years). A different answer for this question is, it may be an effect of a publishing bias. Unfortunately, it could be that 100% of included studies have detected a significant difference because the studies which not detected such that significant difference has no or little chance to be published (publishing bias).

The methodological quality of included studies have no clear effect on their outcomes which means that the ability of the study to detect a significant differences between pesticides exposed and non-exposed workers is not likely to be affected by methodological issues of these studies. Therefore the outcomes of included studies more probably depend on the validity of used biomarkers rather than the methodological quality of study.

Correlation between duration of pesticides exposure and cytogenetic biomarker results has conducted by three included studies Bhalli et al.[15], Zeljezic et al.[16] and Naravaneni et al.[12]. Two of them Bhalli et al.[15] and Zeljezic et al.[16] found a statistically significant association which indicate a possible dose-response relationship between duration of exposure and cytogenetic biomarkers. In other hand Naravaneni et al.[12] reported no significant correlation between duration of exposure and cytogenetic biomarkers. But I think something wrong with results of Naravaneni et al.[12] because these results are contradicting with other results of same study where he reported a significant correlation between duration of exposure and cholinesterase biomarkers and another significant correlation between cholinesterase biomarkers themselves and cytogenetic biomarkers. So how could that happen mathematically without some sort of correlation between duration of exposure and cytogenetic biomarkers?

Cholinesterase biomarkers (E AchE) have found to be correlated with duration of exposure by Naravaneni et al.[12] and Singh et al.[14] These results are consistent with literature. Del Prado Lu and Junky.[20] who postulated that plasma cholinesterase esterase is suitable for measurement of short duration exposure whereas

erythrocyte cholinesterase esterase is suitable for assessment of longer duration of pesticides exposure.

Three included studies have investigated the statistical correlation between cytogenetic and cholinesterase biomarkers. Naravaneni et al [12] and Paz-y-Mino et al.[13] have observed significant correlation between cytogenetic biomarkers (CA and CA"s) and cholinesterase biomarkers (E chE), while Singh et al.[14] reported that no significant correlation found between (CA) and (E chE) biomarkers. Although biomarkers generally work in different mechanisms, it makes sense to have a correlation between tow biomarkers designing to measure the same variable (pesticides toxicity). Furthermore, using of agreement level (Kappa statistic) could be more useful in the assessment of association between cytogenetic and cholinesterase biomarkers. Also, if we had the raw data, we were able to calculate the sensitivities and specificities of biomarkers for each study. Then we could use latent class model for calculation of expected sensitivity and specificity for each biomarker in order to compare them to each other.

CONCLUSION

The cytogenetic biomarkers have more reliability than cholinesterase biomarkers in assessment of chronic toxicity that result from pesticides exposure , also there is an evident of possible association between duration of pesticides exposure and extent of toxic change that occur in those biomarkers especially cytogenetic and (E chE) biomarkers. Therefore the cytogenetic biomarkers may be a good substitute for cholinesterase biomarkers in assessment of pesticides chronic toxicity. Also theoretically, simultaneous use of cytogenetic and cholinesterase biomarkers will increase the net sensitivity of both biomarkers to detect pesticides chronic toxicity among exposed workers.

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